CLAIMS

1. An *in vitro* method of screening human subjects to assess their risk of developing cervical carcinoma, which method comprises screening the subject for expression of mRNA transcripts of the E6 gene of HPV and sorting the subject into one of two categories of risk for development of cervical carcinoma based on expression of E6 mRNA, wherein individuals positive for expression of E6 mRNA are scored as carrying integrated HPV or a modified episomal HPV genome and are therefore classified as high risk for development of cervical carcinoma, whereas individuals negative for expression of E6 mRNA are scored as not carrying integrated HPV or a modified episomal HPV genome and are therefore classified as no detectable risk for development of cervical carcinoma, characterised in that screening for E6 mRNA expression is carried using isothermal amplification in combination with real-time detection of the amplification product.

2. An *in vitro* method of identifying human subjects having abnormal cell changes in the cervix, which method comprises screening the subject for expression of mRNA transcripts of the E6 gene of HPV, wherein individuals positive for expression of E6 mRNA are identified as having abnormal cell changes in the cervix, characterised in that screening for E6 mRNA expression is carried using isothermal amplification in combination with real-time detection of the amplification product.

3. A method according to claim 1 or claim 2 wherein the isothermal amplification is NASBA, transcription-

mediated amplification, signal-mediated amplification of RNA or isothermal solution phase amplification.

4. A method according to claim 3 wherein screening for E6 mRNA expression is carried out using real-time NASBA.

5. A method according to any one of claims 1 to 4 wherein the human subjects are subjects previously identified as infected with human papillomavirus DNA in cells of the cervix.

6. A method according to any one of claims 1 to 5 wherein the human subjects are subjects having a previous diagnosis ASCUS, CIN 1 lesions or condyloma.

7. A method according to any one of claims 1 to 6 which comprises screening for E6 mRNA expression using a technique which is able to detect E6 mRNA from at least one cancer-associated HPV type.

8. A method according to claim 7 which comprises screening for E6 mRNA expression using a technique which is able to detect E6 mRNA from HPV types 16, 18, 31, 33, and preferably 45.

9. A method according to any one of claims 1 to 8 wherein individuals positive for expression of E6 mRNA from at least one of HPV types 16, 18, 31, 33 or 45 are scored as carrying integrated HPV.

10. A kit for use in the detection of mRNA transcripts of the E6 gene(s) of HPV, the kit comprising one or more

primer-pairs which enable amplification of a region of E6 transcripts from HPV types 16, 18, 31 and 33 by NASBA and one or more molecular beacon probes.

11. A kit according to claim 10 which comprises separate primer-pairs specific for each of HPV types 16, 18, 31 and 33.

12. A kit according to claim 10 or claim 11 which comprises one or more of, two or more of and preferably all of the following primer pairs and accompanying identification probes:
5' gatgcaaggtcgcatatgagCCACAGGAGCGACCCAGAAA and 5' AATTCTAATACGACTCACTATAGGGAGAAGGATTCCCATCTCTATATACTA with probe TATGACTTTGCTTTTCGGGA

5' gatgcaaggtcgcatatgagGAAAACGATGAAATAGATGGAG and 5' AATTCTAATACGACTCACTATAGGGAGAAGGGGTCGTCTGCTGAGCTTTCT with probe GAACCACAACGTCACACAATG

5' gatgcaaggtcgcatatgagACTGACCTCCACTGTTATGA and 5' AATTCTAATACGACTCACTATAGGGAGAAGGTATCTACTTGTGTGCTCTGT with probe GGACAAGCAGAACCGGACACATCCAA

5' GATGCAAGGTCGCATATGAGTATCCTGAACCAACTGACCTAT and 5' AATTCTAATACGACTCACTATAGGGAGAAGGTTGACACATAAACGAACTG with probe GGACAAGCACAACCAGCCACAGC.

554059; NLW; NLW